AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Please delete the paragraph on page 1, lines 3-11 before "Background of the Invention", and replace with the following cross referencing information:

> This application is a continuation of U.S. application Serial No. 10/015,208, filed November 16, 2001, now U.S. Patent 6,699,458, which is a continuation of U.S. application Serial No. 09/778,969, filed February 5, 2001, now U.S. Patent No. 6,359,120, which is a continuation of U.S. Serial No. 08/473,562 filed on June 6, 1995, now U.S. Patent No. 6,184,361, which is a divisional of U.S. Serial No. 08/415,743 filed April 3, 1995, now U.S. Patent No. 5,808,091, which is a continuation of U.S. Serial No. 08/054,120 filed April 27, 1993, now abandoned, which is a continuation-in-part of U.S. Serial No. 07/976,079 filed November 13, 1992, now abandoned, which is a continuation-in-part of U.S. Serial No. 07/784,486 filed October 29, 1991, now abandoned, all of which are hereby incorporated by reference.

Please delete the sections entitled "Background of the Invention" and "Brief Description of the Invention" (which begin at page 1, line 8, and end at page 10, line 18), and replace such deleted sections with the following replacement sections:

Background of the Invention

Many of the procedures presently conducted in the field of nuclear medicine involve radiopharmaceuticals which provide diagnostic images of blood flow (perfusion) in the major organs and in tumors. The regional uptake of these radiopharmaceuticals

within the organ of interest is proportional to flow; high flow regions will display the highest concentration of radiopharmaceutical, while regions of little or no flow have relatively low concentrations. Diagnostic images showing these regional differences are useful in identifying areas of poor perfusion, but do not provide metabolic information of the state of the tissue within the region of apparently low perfusion.

There is a need for new radiopharmaceuticals which specifically localize in hypoxic tissue, i.e., tissue which is deficient in oxygen, but still viable. These compounds should be retained in regions which are hypoxic, but should not be retained in regions which are normoxic. A radiopharmaceutical with these properties will display relatively high concentrations in such hypoxic regions, with low concentrations in normoxic and infarcted regions. Diagnostic images with this radiopharmaceutical should readily allow the identification of tissue which is at risk of progressing to infarction, but still salvagable in, for example, the heart and brain.

It is well known that tumors often have regions within their mass which are hypoxic. These result when the rapid growth of the tumor is not matched by the extension of tumor vasculature. A radiopharmaceutical which localizes preferentially within regions of hypoxia could also be used to provide images which are useful in the diagnosis and management of therapy of tumors as suggested by Chapman, "Measurement of Tumor Hypoxia by Invasive and Non-Invasive Procedures — A Review of Recent Clinical Studies", Radiother. Oncol., 20(S1), 13-19 (1991). Additionally, a compound which localizes within the hypoxic region of tumors, but is labeled with a radionuclide with suitable α- or β-emissions could be used for the internal radiotherapy of tumors.

As reported by Martin at al. ("Enhanced Binding of the Hypoxic Cell Marker [^{3}H] Fluoromisonidazole", J. Nucl. Med., Vol. 30, No. 2, 194-201 (1989)) and Hoffman et al. ("Binding of the Hypoxic Tracer [H-3] Misonidazole in Cerebral Ischemia", Stroke, Vol. 18, 168 (1987)), hypoxia-localizing moieties, for example, hypoxia-mediated nitroheterocyclic compounds (e.g., nitroimidazoles and derivatives thereof) are known to be retained in hypoxic tissue. In the brain or heart, hypoxia typically follows ischemic episodes produced by, for example, arterial occlusions or by a combination of increased demand and insufficient flow. Additionally, Koh et al., ("Hypoxia Imaging of Tumors Using [F-18] Fluoronitroimidazole", J. Nucl. Med., Vol. 30, 789 (1989)) have attempted diagnostic imaging of tumors using a nitroimidazole radiolabeled with ^{18}F. A nitroimidazole labeled with ^{123}I has been proposed by Biskupiak et al. ("Synthesis of an (iodovinyl)misonidazole derivative for hypoxia imaging", J. Med. Chem., Vol. 34, No. 7, 2165-2168 (1991)) as a radiopharmaceutical suitable for use with single-photon imaging equipment.

While the precise mechanism for retention of hypoxia-localizing compounds is not known, it is believed that nitroheteroaromatic compounds, such as misonidazole, undergo intracellular enzymatic reduction (for example, J. D. Chapman, "The Detection and Measurement of Hypoxic Cells in Tumors", Cancer, Vol. 54, 2441-2449 (1984)). This process is believed to be reversible in cells with a normal oxygen partial pressure, but in cells which are deficient in oxygen, further reduction can take place. This leads to the formation of reactive species which bind to or are trapped as intracellular components, providing for preferential entrapment in hypoxic cells. It is necessary, therefore, for hypoxia imaging compounds to possess certain specific properties; they

must be able to traverse cell membranes, and they must be capable of being reduced, for example, by reductases such as xanthine oxidase.

The hypoxia imaging agents mentioned above are less than ideal for routine clinical use. For example, the positron-emitting isotopes (such as ^{18}F) are cyclotron-produced and short-lived, thus requiring that isotope production, radiochemical synthesis, and diagnostic imaging be performed at a single site or region. The costs of procedures based on positron-emitting isotopes are very high, and there are very few of these centers worldwide. While ^{123}I-radiopharmaceuticals may be used with widely-available gamma camera imaging systems, ^{123}I has a 13 hour half-life (which restricts the distribution of radiopharmaceuticals based on this isotope) and is expensive to produce. Nitroimidazoles labeled with ^{3}H are not suitable for <u>in vivo</u> clinical imaging and can be used for basic research studies only.

The preferred radioisotope for medical imaging is ^{99m}Tc. Its 140 keV γ-photon is ideal for use with widely-available gamma cameras. It has a short (6 hour) half life, which is desirable when considering patient dosimetry. ^{99m}Tc is readily available at relatively low cost through commercially-produced $^{99}Mo/^{99m}Tc$ generator systems. As a result, over 80% of all radionuclide imaging studies conducted worldwide utilize this radioisotope. To permit widespread use of a radiopharmaceutical for hypoxia imaging, it is necessary that the compound be labeled with ^{99m}Tc. For radiotherapy, the rhenium radioisotopes, particularly ^{186}Re and ^{188}Re, have demonstrated utility.

EP 411,491 discloses boronic acid adducts of rhenium dioxime and technetium-99m dioxime complexes linked to various nitroimidazoles. Although these complexes are believed to be useful for diagnostic and therapeutic purposes, it would be desirable to

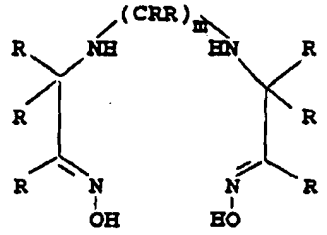
obtain higher levels of the rhenium or technetium radionuclide in the targeted area, than are achieved with this class of capped-dioxime nitroimidazole complexes. It was demonstrated that the compounds disclosed in EP 411,491 possess reduction potentials similar to 2-nitroimidazole derivatives known to localize in hypoxic regions. In addition, the reduction of these compounds is catalyzed by xanthine oxidase. However, these compounds have poor membrane permeability. Thus, while these compounds might be retained by hypoxic cells, delivery of these compounds to the intracellular domain of these cells may be less than ideal. In addition, the complexes described in EP 411,491 require a heating step to form the hypoxialocalizing radiolabeled compounds. It would be more convenient for the routine use of such hypoxia-localizing radiolabeled compounds to be able to prepare such complexes at ambient temperatures.

Radiolabeled complexes of hypoxia-localizing moieties which retain the biochemical behavior and affinity of such moieties, which are labeled at room temperature with a suitable, easy-to-use radionuclide, and which are capable of providing increased amounts of the desired radionuclide to the targeted area, would be a useful addition to the art.

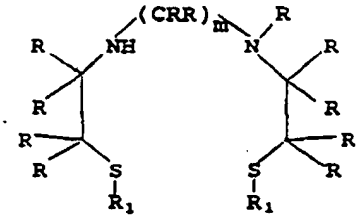
Brief Description of the Invention

In accordance with the present invention, novel ligands, metal complexes of such ligands, processes for their preparation, and diagnostic and therapeutic methods for their use, are disclosed. In particular, metal complexes, e.g., technetium and rhenium complexes, which are linked to a hypoxia localizing moiety, and wherein the complex has a permeability through cell membranes greater than that of ^{14}C-sucrose, are disclosed. Exemplary complexes are useful as diagnostic imaging agents in the case of technetium radionuclides and improved agents for radiotherapy in the case of rhenium radionuclides.

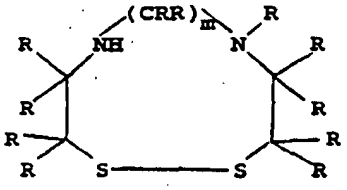
Suitable novel ligands to form these complexes may include, but are not limited to, di-, tri- or tetradentate ligands forming neutral complexes of technetium or rhenium with the metal preferably in the +5 oxidation state. Examples of such ligands are represented by the formulae

Ia 

or

Ib 

or

Ic 

where at least one R is $-(A)_p-R_2$ where $(A)_p$ is a linking group and R_2 is a hypoxia localizing moiety; and wherein the other R groups are the same, or different and are independently selected from hydrogen, halogen, hydroxy, alkyl, alkenyl, alkynyl, alkoxy, aryl, $-COOR_3$, $\overset{O}{\overset{\|}{C}}-NHR_3$, $-NH_2$, hydroxyalkyl, alkoxyalkyl, hydroxyaryl, haloalkyl, arylalkyl, $-alkyl-COOR_3$, $-alkyl-CON(R_3)_2$, $-alkyl-N(R_3)_2$, $-aryl-COOR_3$, $-aryl-CON(R_3)_2$,

-aryl-$N(R_3)_2$, 5- or 6-membered nitrogen- or oxygen containing heterocycle; or two R groups taken together with the one or more atoms to which they are attached form a carbocyclic or heterocyclic, saturated or unsaturated spiro or fused ring which may be substituted with R groups;

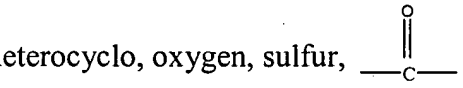
R_1 is hydrogen, a thiol protecting group or $-(A)_p-R_2$;

R_3 is hydrogen, alkyl or aryl;

m = 2 to 5; and,

p = 0 to 20.

It should be apparent that the disulfide of Ic can be reduced to the corresponding dithiol of Ib by known methodology prior to complexing with a metal.

The linking group $(A)_p$ can be any chemical moiety which can serve to physically distance, or otherwise isolate, the hypoxia localizing moiety from the rest of the complex of formula I. This might be important if the hypoxia localizing moiety is likely to be inhibited in its action by the rest of the complex. For example, in the linking group, wherein p is one, A, or the various A units in forming a straight or branched chain if p > 1, are independently selected from $-CH_2-$, $-CHR_4-$, $-CR_4R_5-$, -CH=CH-, $-CH=CR_4-$, $-CR_4-CR_5-$, -C=C-, cycloalkyl, cycloalkenyl, aryl, heterocyclo, oxygen, sulfur,  , -NH-, -HC=N-, $-CR_4=N-$, -NR,-, -Cs-; wherein R_4 and R_5 are independently selected from alkyl, alkenyl, alkoxy, aryl, 5- or 6-membered nitrogen or oxygen containing heterocycle, halogen, hydroxy or hydroxyalkyl.

In considering the various linking groups known in the art, it is understood that p could be any convenient value depending upon the design choices for the desired complex. Preferably, p is ≤ 20 and most preferably $p \leq 10$.

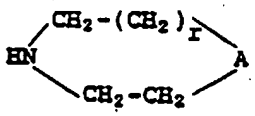
Listed below are definitions of the terms used to describe the complexes of this invention. These definitions apply to the terms as they are used throughout the specification (unless they are otherwise limited in specific instances) either individually or as part of a larger group.

The terms "alkyl", "alkenyl" and "alkoxy" refer to both straight and branched chain groups. Those groups having 1 to 10 carbon atoms are preferred.

The term "aryl" refers to phenyl and substituted phenyl. Preferred are phenyl and phenyl substituted with 1, 2 or 3 alkyl, haloalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, alkoxy, alkoxyalkyl, halogen, amino, hydroxy, or formyl groups.

The terms "halide", "halo" and "halogen" refer to fluorine, chlorine, bromine and iodine.

The expression "5- or 6-membered nitrogen containing heterocycle" refers to all 5- and 6-membered rings containing at least one nitrogen atom. Exemplary aliphatic nitrogen heterocyclic derivatives have the formula



wherein r is 0 or 1 and A is -O-, -N-R_6, -S- or -CH-R_6 wherein R_6 is hydrogen, alkyl, aryl or arylalkyl. Such groups include pyrrolidinyl, piperidinyl, morpholinyl, piperazinyl, 4-alkylpiperazinyl, 4-alkylpiperidinyl, and 3-alkylpyrrolidinyl groups. Also included within the expression "5- or 6-membered nitrogen containing heterocycle" are aromatic groups. Exemplary aromatic groups are pyrrolyl, imidazolyl, oxazolyl, pyrazolyl, pyridinyl, thiophenyl, pyridazinyl, thiazolyl, triazolyl and pyrimidinyl groups. The above groups can be linked via a hetero atom or a carbon atom.

The expression "5- or 6-membered nitrogen or oxygen containing heterocycle" refers to all sand 6-membered rings containing at least one nitrogen or oxygen atom. Exemplary groups are those described above under the definition of the expression "5- or 6-membered nitrogen containing heterocycle". Additional exemplary groups are 1,4-dioxanyl and furanyl.